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Short communication

Evaluation of the acute phase response in cloned pigs following a lipopolysaccharide challenge

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Abstract

The objective of this study was to evaluate the acute phase response (APR) in cloned pigs derived from two different cell lines [C1 ($n = 2$) and C2 ($n = 7$)] as compared to genetically similar non-cloned pigs (CONT; $n = 11$) following a lipopolysaccharide (LPS; 25 μg/kg BW) challenge. Pigs were weaned at 21 days of age and maintained in individual pens in the same room until sample collection approximately 1 week later. Blood samples were collected every 30 min for 2 h prior to and 4 h after the LPS challenge. Serum samples were analyzed for cortisol, tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6). Average gestational length for cloned pigs, 118.8 ± 0.97 days, was longer ($P < 0.005$) than that of CONT pigs, 114 ± 0.41 days. For serum cortisol, there was a time by group interaction ($P < 0.0001$) such that the cortisol response was greater in CONT pigs as compared to C2 pigs ($P < 0.0001$), but not different from C1 pigs ($P > 0.74$). A time by group interaction ($P < 0.0001$) was observed for serum TNF-α such that the TNF-α response was greater in CONT pigs as compared to C2 pigs ($P = 0.0002$) and tended to be greater ($P < 0.06$) than C1 pigs. A time by group interaction ($P < 0.0001$) was also observed for serum IL-6 such that the serum IL-6 response was greater ($P < 0.003$) in CONT pigs as compared to C2 pigs and there was a trend ($P = 0.10$) for serum IL-6 to be greater in CONT pigs compared to

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the C1 pigs. These are the first results to demonstrate that cortisol and proinflammatory cytokine profiles associated with the APR of cloned pigs are altered compared to genetically similar non-cloned pigs. Our results also indicate that the cell line from which clones are derived may dictate the APR. The hormone and cytokine profiles reported herein are a significant contribution towards our understanding, and perhaps our ability to prevent or reduce the incidence of premature deaths in cloned animals and warrants further investigation of the immune system of cloned animals.
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Keywords: Clones; Pigs; Acute phase response

1. Introduction

Cloning by somatic cell nuclear transfer (SCNT) is currently the leading method for the production of both transgenic and knockout large animal species. Genetically modified animals have been developed that serve as bioreactors, biomedical research models and as potential organ donors for xenotransplantation [1]. While numerous reports have detailed that cloned animals are normal, perinatal and early postnatal death losses still appear to be higher than in control animals. To date, these perinatal and early postnatal deaths have been attributed to a plethora of abnormalities including but not limited to metabolic and cardiopulmonary abnormalities [2–4], lymphoid hypoplasia [5] and neonatal respiratory distress [3]. Additionally, other reports have indicated that cloned calves [3], lambs [6], goats [7] and piglets [8,9] have died of bacterial infections and sudden death of unknown causes in the neonatal period. Unfortunately, there remains a rather large void in the available information concerning the details surrounding the early deaths of cloned animals [2–5,9–16]. While these early deaths in cloned animals have been attributed to numerous anomalies, a previous report on a cloned calf that died at 51 days which revealed decreased circulating lymphocytes, thymic atrophy and lymphoid hypoplasia [5] suggests that abnormalities may exist in the functionality of the immune system of cloned animals. Previously, in an effort to evaluate the adaptive immune response in cloned pigs, we demonstrated that cloned piglets responded adequately to killed vaccines, produced normal levels of IgG and IgM and had normal populations of CD4+, CD8+ and CD4+ 8+ lymphocyte counts [8]. However, given the increased incidence of death in cloned animals during the perinatal and early postnatal period attributed to bacterial infections, it would be reasonable to assume that the acute phase response (APR), an innate body defense, rather than the adaptive immune system, of cloned animals may be altered. While an animal's innate immunity does not recognize all possible antigens, as does the adaptive immunity, it does recognize highly conserved structures in many different microorganisms, known as pathogen-associated molecular patterns which elicit the APR in the body. A well-known pathogen-associated molecular pattern is that of lipopolysaccharide (LPS) from the cell wall of Gram-negative bacteria. Therefore, the objective of this study was to evaluate the APR, as indicated by some critical but subtle aspects of the APR or host reaction to its mediators, of apparently normal cloned piglets following an LPS challenge.

2. Materials and methods

2.1. Animals

Data from nine genetically identical cloned piglets derived from two different cell lines [Cell line 1 (C1; $n = 2$ pigs from one litter) and Cell line 2 (C2; $n = 7$ pigs from four litters)] and 11 control piglets (CONT) was collected as part of this study. Five litters of cloned piglets were delivered by natural delivery with an average gestational length of 118.8 ± 0.97 days compared to 114 ± 0.41 days for the four litters of CONT pigs. Given that C1 pigs represented only one litter, average gestational length for the cloned pigs was calculated as the average gestational length for all cloned pigs. It should be noted that the gestational length for the two C1 pigs from the single litter was 116 days. The nuclear transfer techniques used to create the cloned piglets have been described in detail elsewhere [17–19]. The fibroblast cell lines used as donor cells for making the cloned piglets were derived from 2-day 35 porcine fetuses that were full sibs from the same litter obtained from the University of Missouri swine production facility. These cells were on passage 3 and cultured for 12 days after the initial isolation of the cells. Activation of the reconstructed embryos was accomplished by using either thimersol or an electrical current, and cell membrane fusion was accomplished by using an electrical current [20]. Control piglets were the result of natural pregnancies obtained from the same genetically similar herd. All clone and CONT pig litters were delivered naturally and raised in the same room under the same environmental conditions in a facility compliant with all regulations set forth in the *Guide for the Care and Use of Laboratory Animals.*

Both groups were weaned at 21 days of age and maintained in individual pens in the same experimental room until the time of sample collection at 27–30 days of age. One day prior to the challenge, pigs were fitted non-surgically with jugular catheters as described by Carroll et al. [21]. All pigs were weighed on the day of catheterization to calculate the dose of lipopolysaccharide. The following day, pigs were challenged i.v. with 25 μg/kg of body weight with LPS *(Escherichia coli* serotype 0111: B4; Sigma L-2630, Sigma Chemical, St. Louis, MO) dissolved in 0.9% (w/v) NaCl solution immediately following collection of the blood sample at Time 0. Blood samples were collected every 30 min via the jugular catheter from −2 to 4 h relative to challenge. Serum was harvested from blood samples and stored at −80 °C until later analysis.

2.2. Hormone and cytokine analysis

For hormone and cytokine analysis, samples were analyzed in duplicate within a single assay. Serum concentration of cortisol was determined using a Coat-a-Count assay kit (Diagnostic Products), which we previously validated in our laboratory [21]. The dynamic range was 5–500 ng/mL with a minimum detectable dose of 2 ng/mL with an intra-assay coefficient of variation of 3.68%. Serum concentration of tumor necrosis factor-alpha (TNF-α) was determined using a porcine specific TNF-α ELISA kit as per the instructions of the manufacturer (Quantikine PTA00, R&D Systems, Minneapolis, MN). The dynamic range of the assay was 23.4–1500 pg/mL with a minimum detectable dose ranging from 2.8 to 5.0 pg/mL. The intra-assay coefficient of variation was 2.16%. Serum concentration of interleukin-6

(IL-6) was analyzed using a commercially available ELISA kit specific for porcine IL-6 (Quantikine P6000, R&D Systems, Minneapolis, MN). Analyses were conducted as outlined by the manufacturer. The dynamic range of the assay was 39.1–2500 pg/mL with an intra-assay coefficient of variation of 2.33%. The minimum detectable dose of porcine IL-6 for this kit is typically 10 pg/mL.

2.3. Statistical analysis

Statistical analyses were performed using Statview software (SAS Inst. Inc., Cary, NC). For gestational length comparison, gestation lengths for Cell lines 1 and 2 pigs were combined due to the limited number of litters represented in Cell line 1 ($n = 1$), resulting in a total of five litters of cloned pigs and four litters of control pigs. Gestational length, treatment ages and treatment body weight comparisons were analyzed using a factorial ANOVA with genotype (cloned versus control) as the main effect. When significant main effects existed ($P < 0.05$), specific group comparisons were generated using Fisher's Protected least significant difference. Serum cortisol, TNF-α and IL-6 were analyzed by analysis of variance specific for repeated measures. The statistical model for serum cortisol and cytokines included the effects of genotype (Cell lines 1, 2 and control), time and interactions. Specific time point comparisons were analyzed using a paired *t*-test comparison. *P*-Values equal to or less than 0.05 were considered significant. Data are expressed as the mean ± standard error of the mean.

3. Results

The average gestational length for the cloned piglets (C1 and C2 combined), 118.8 ± 0.97 days, was longer ($P = 0.0043$) than that of the CONT piglets, 114 ± 0.41 days. For purposes of this report, gestational length is defined as the number of days from the day of ovulation for the surrogate to the day of parturition. The ages of the pigs on the day of the LPS challenge did not differ ($P > 0.10$) among the groups, and were 30.0 ± 0.0, 28.3 ± 0.8 and 26.6 ± 0.2 days of age for the C1, C2 and CONT pigs, respectively. Body weights of the pigs at the time of treatment were also not different ($P > 0.06$) among the three groups of pigs and averaged 6.98 ± 0.53, 6.05 ± 0.50 and 7.59 ± 0.38 kg for the C1, C2 and CONT pigs, respectively.

During the pre-LPS challenge period, blood samples were collected to evaluate basal cortisol and cytokine profiles in both groups of pigs. There was no time by group interaction ($P > 0.59$) for serum concentrations of cortisol or TNF-α during the pre-LPS period (i.e., −2 to 0 h), therefore, basal concentrations of cortisol (Fig. 1) and TNF-α (Fig. 2) are reported as the mean serum concentration from −2 to 0 h. Basal serum concentration of cortisol was 6-fold lower ($P = 0.0004$) in the C1 clones and 1.47-fold lower ($P < 0.03$) in the C2 clones as compared to the CONT pigs (27.62 ± 2.97 ng/mL). Additionally, basal serum concentration of cortisol was lower ($P < 0.04$) in the C1 clones (4.6 ± 0.62 ng/mL) as compared to the C2 clones (18.8 ± 2.29 ng/mL). Basal serum concentration of TNF-α was 4-fold lower ($P < 0.007$) in the C1 clones and 2.7-fold lower ($P = 0.0005$) in the C2 clones as compared to the CONT pigs (0.16 ± 0.022 ng/mL). Basal serum concentration of

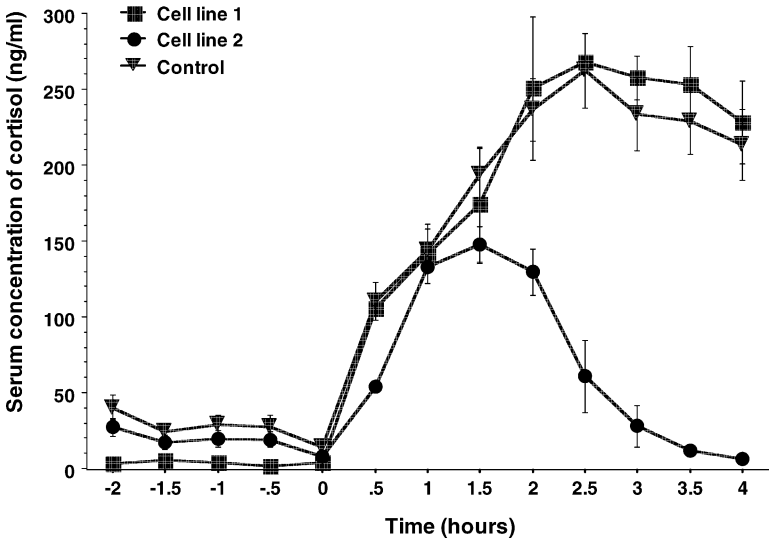

Fig. 1. Serum concentration of cortisol in two cell lines of cloned pigs (Cell lines 1 and 2) and control pigs 2 h prior to (basal) and 4 h following a lipopolysaccharide (LPS; 25 μg/kg body weight) challenge. Basal serum concentration of cortisol was lower in the Cell line 1 clones ($P = 0.0004$) and Cell line 2 clones ($P < 0.03$) as compared to the control pigs. Additionally, basal serum concentration of cortisol was lower ($P < 0.04$) in the Cell line 1 clones as compared to the Cell line 2 clones. Following the LPS challenge at Time 0, there was a time by group interaction ($P < 0.0001$) such that the cortisol response was greater in control pigs ($P < 0.0001$) and Cell line 1 clones ($P < 0.002$) as compared to the Cell line 2 clones. The serum cortisol response did not differ ($P > 0.86$) between the control pigs and Cell line 1 clones.

TNF-α did not differ ($P > 0.63$) between the C1 clones (0.04 ± 0.007 ng/mL) and C2 clones (0.06 ± 0.006 ng/mL). For serum IL-6, however, basal serum concentrations were below the minimum detectable dose (10 pg/mL) for all pigs (Fig. 3).

Following the LPS challenge at Time 0, blood samples were collected to evaluate the acute phase response to an endotoxin challenge (i.e., LPS) as indicated by serum concentrations of cortisol, TNF-α and IL-6. While serum concentrations of cortisol, TNF-α and IL-6 each increased in all groups of pigs following the LPS injection, the response curves were dramatically different.

For serum cortisol there was a time by group interaction ($P < 0.0001$) such that the cortisol response was greater in CONT ($P < 0.0001$) and C1 ($P < 0.002$) pigs as compared to the C2 clones (Fig. 1). The serum cortisol response did not differ ($P > 0.86$) between the CONT pigs and C1 clones. Initially, there was over a 2.9-fold increase ($P < 0.0001$) in all groups of pigs within 30 min post-LPS injection. In the CONT pigs and C1 clones, serum cortisol concentrations continued to increase until reaching peaks of 263.13 ± 24.47 and 268.9 ± 1.7 ng/mL, respectively, at 2.5 h post-LPS, whereas in the C2 clones, serum cortisol concentrations reached a peak of 148.06 ± 12.37 ng/mL at 1.5 h post-LPS. At 4 h post-LPS injection, cortisol concentration in the CONT pigs and C1 clones remained elevated and was over 14-fold greater ($P < 0.0001$) than serum concentration of cortisol at Time 0 prior to the LPS injection. In the C2 clones, serum cortisol concentration at 4 h post-

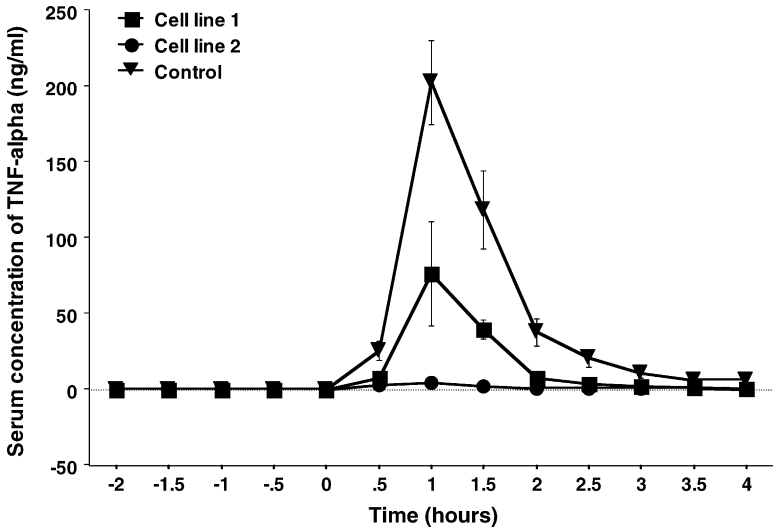

Fig. 2. Serum concentration of tumor necrosis factor-alpha (TNF-α) in two cell lines of cloned pigs (Cell lines 1 and 2) and control pigs 2 h prior to (basal) and 4 h following a lipopolysaccharide (LPS; 25 μg/kg body weight) challenge. Basal serum concentration of TNF-α was 4-fold lower ($P < 0.007$) in the Cell line 1 clones and 2.7-fold lower ($P = 0.0005$) in the Cell line 2 clones as compared to the control pigs. Basal serum concentration of TNF-α did not differ ($P > 0.63$) between the Cell line 1 clones and Cell line 2 clones. Following the LPS challenge at Time 0, a time by group interaction ($P < 0.0001$) was observed for serum TNF-α. Serum concentrations of TNF-α remained above Time 0 concentrations for both the control pigs and the Cell line 2 clones at 4 h post-LPS injection ($P < 0.0007$), and were not different ($P > 0.23$) from Time 0 for the Cell line 1 clones.

LPS injection was not different ($P > 0.27$) from serum concentration of cortisol at Time 0.

As with serum cortisol, a time by group interaction ($P < 0.0001$) was observed for serum TNF-α (Fig. 2). By 30 min post-LPS injection, serum concentrations of TNF-α had increased ($P < 0.003$) in all groups of pigs. At 1 h post-LPS, serum concentration of TNF-α was over 2.6-fold greater ($P < 0.04$) in the CONT pigs as compared to the C1 clones, and over 45.2-fold greater ($P < 0.0001$) in the CONT pigs as compared to the C2 clones. Serum concentrations of TNF-α peaked in the CONT pigs (202.76 ± 27.94 ng/mL), C1 clones (76.87 ± 34.32 ng/mL) and C2 clones (4.48 ± 1.04 ng/mL) pigs at 1 h post-LPS injection. Serum concentrations of TNF-α remained above Time 0 concentrations for both the CONT pigs and the C2 clones at 4 h post-LPS injection ($P < 0.0007$), and were not different ($P > 0.23$) from Time 0 for the C1 clones. At the 4 h time point, serum concentrations of TNF-α were 17.8-fold greater in CONT pigs as compared to the C2 clones.

Similar to the serum response curves for cortisol and TNF-α, a time by group interaction ($P < 0.0001$) was observed for serum IL-6 (Fig. 3). In the CONT pigs, serum concentration of IL-6 was increased ($P < 0.04$) by 30 min post-LPS injection compared to Time 0 and continued to increase until reaching a peak of $10{,}758.14 \pm 2197.1$ pg/mL at 2.5 h post-LPS injection. In the C1 clones and C2 clones, serum concentrations of IL-6 did not increased above Time 0 concentrations until 1 h post-LPS injection. Serum concentration of IL-6 continued to increase in the C1 clones until reaching a peak of 2709.6 ± 448.02 pg/mL at 2 h

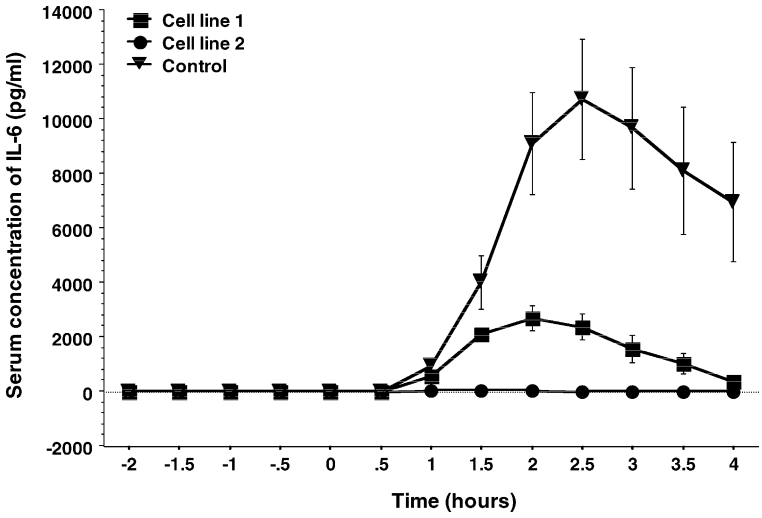

Fig. 3. Serum concentration of interleukin 6 (IL-6) in two cell lines of cloned pigs (Cell lines 1 and 2) and control pigs 2 h prior to (basal) and 4 h following a lipopolysaccharide (LPS; 25 μg/kg body weight) challenge. Basal serum concentration of IL-6 was below the detection limit of the ELISA (10 pg/mL) in both lines of cloned pigs and control pigs. Following the LPS challenge at Time 0, a time by group interaction ($P < 0.0001$) was observed for serum IL-6. In the control pigs, serum concentration of IL-6 was increased ($P < 0.04$) by 30 min post-LPS injection compared to Time 0 and continued to increase until reaching a peak at 2.5 h post-LPS injection. In the Cell line 1 clones and Cell line 2 clones, serum concentrations of IL-6 did not increased above Time 0 concentrations until 1 h post-LPS injection.

post-LPS. In the C2 clones, however, serum concentrations of IL-6 reached a peak of only 43.28 ± 13.36 pg/mL at 1.5 h post-LPS injection. After the 3 h time point, serum concentration of IL-6 was no longer detectable in the C2 clones. However, in the C1 clones, serum concentration of IL-6 did not return to basal concentration until 4 h post-LPS injection. In the CONT pigs, serum concentrations of IL-6 remained elevated ($P < 0.01$) above Time 0 concentrations out to 4 h post-LPS injection.

4. Discussion

Knowing the complicated nature of the cloning process, it is easy to believe that there would be a high incidence of birth defects and mortality in cloned offspring. Reports have indicated that the postnatal survival of cloned calves can be as low as 33% in some studies [22], and we have experienced similar losses in cloned piglets at the University of Missouri [8]. However, there exists few published health reports of animals created through nuclear transfer. While complete blood counts (CBC) and clinical chemistries have been measured in cloned calves and piglets, these indices show very few differences when compared to control animals. Various hormonal studies have also been performed on cloned calves. These have reported clones to have decreased plasma thyroxine (T_4) concentrations, higher plasma leptin concentrations and normal plasma growth hormone concentrations. Additionally, the

response of insulin and blood glucose after feeding, as well as the response to ACTH stimulation testing, were also found to be normal in these cloned calves [23]. However, as in the present study with cloned pigs from two different cell lines, basal concentrations of cortisol have been reported to be lower in cloned calves [23]. Given that the majority of glucocorticoid activity in domestic animals such as sheep, pigs and cattle is from cortisol, and the fact that glucocorticoids are known to have multiple effects on fetal development and immune function, as well as neonatal metabolism and growth, one might suspect that lower basal cortisol in cloned pigs and calves could have implications with regard to perinatal and early postnatal survival and well-being. However, the exact cause of this apparent abnormality in the acute phase response of the cloned pigs utilized in the current study is not known at this time, and one should consider that there are several epigenetic mechanisms that could have contributed to the altered acute phase responses observed in the present study.

To our knowledge, this is the first study to report the cortisol, TNF-α and IL-6 profiles associated with the acute phase response following an endotoxin challenge in any cloned species. The present results should serve as a valid launching point to support further research into the nature of the variability within the observed hormone and cytokine responses of cloned animals. It should be noted that the inadequacy observed in the acute phase response of the cloned pigs utilized in the current study may reflect a deficiency associated with the specific donor cell lines used to generate these clones, and that cloned pigs derived from a different donor cell line may not exhibit the same inadequacy in immune function. Indeed, our own observations between Cell lines 1 and 2 clones evaluated in the present study strongly suggest that the cell line from which cloned animals are generated may dictate the acute phase response to an immune challenge. These differences reported between the two donor cell lines my also explain the differences found in initial pregnancy rates of recipients carrying nuclear transfer embryos from different cell lines [18]. The authors acknowledge the potential implications associated with the low number of pigs ($n = 2$) which represent Cell line 1 in the current study. However, the inclusion of this data brings to the forefront the fact that cloned pigs, while generated from genetically similar donor cells, can themselves be very different from each other. Regardless of whether these observations are specific to the particular cell lines utilized in the current study or to cloned animals in general, these results warrant further investigation and a more in depth evaluation and testing of the acute phase response in all species of cloned animals.

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References

[1] Prather RS, Hawley RJ, Carter DB, Lai L, Greenstein JL. Transgenic swine for biomedicine and agriculture. Theriogenology 2002;8793:1–9.

[2] Cibelli JB, Stice SL, Golueke PJ, Kane JJ, Jerry J, Blackwell C, et al. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. Science 1998;280:1256–8.
[3] Hill JR, Roussel AJ, Cibelli JB, Edwards JF, Hooper NL, Miller MW, et al. Clinical and pathological features of cloned transgenic calves and fetuses (13 case studies). Theriogenology 1999;51:1451–65.
[4] Wells DN, Misica PM, Tervit HR. Production of cloned calves following nuclear transfer with cultured adult mural granulosa cells. Biol Reprod 1999;60:996–1005.
[5] Renard JP, Chastant S, Chesne P, Richard C, Marchal J, Cordonnier N, et al. Lymphoid hypoplasia and somatic cloning. Lancet 1999;353:1489–91.
[6] Peura TT, Kleemann DO, Rudiger SR, Nattrass GS, McLaughlan CJ, Walker SK. Effect of nutrition of oocyte donor on the outcomes of somatic cell nuclear transfer in the sheep. Biol Reprod 2003;68:45–50.
[7] Keefer CL, Baldassarre H, Keyston R, Wang B, Bhatia B, Bilodeau AS, et al. Generation of dwarf goat (*capra hircus*) clones following nuclear transfer with transfected and nontransfected fetal fibroblasts and in vitro-matured oocytes. Biol Reprod 2001;64:849–56.
[8] Carter DB, Lai L, Park K-W, Samuel M, Lattimer JC, Jordan KR, et al. Phenotyping of transgenic cloned piglets. Cloning Stem Cells 2002;4:131–45.
[9] Boquest AC, Grupen CG, Harrison SJ, McIlfatrick SM, Ashman RJ, d'Apice AJF, et al. Production of cloned pigs from cultured fetal fibroblast cells. Biol Reprod 2002;66:1283–7.
[10] Behboodi E, Anderson GB, BonDurant RH, Cargill SL, Kreuscher BR, Medrano JF, et al. Birth of large calves that developed from in vitro-deried bovine embryos. Theriogenology 1995;44:227–32.
[11] Denning C, Burl S, Bracken J, Dinnyes A, Fletcher J, King T, et al. Deletion of the a 1,3 galactosyl transferase (GGTA1) gene and the prion protein (PrP) gene in sheep. Nat Biotechnol 2001;19:559–62.
[12] Garry FB, Adams R, McCann JP, Odde KG. Postnatal characteristics of calves produced by nuclear transfer cloning. Theriogenology 1996;45:141–52.
[13] Kato Y, Tani T, Tsunoda Y. Cloning of calves from various somatic cell types of male and female adult, newborn and fetal cows. J Reprod Fertil 2000;120:231–7.
[14] Govoni KE, Tian XC, Kazmer GW, Taneja M, Enright BP, Rivard AL, et al. Age-related changes in the somatotropic axis in cloned holstein calves. Biol Reprod 2002;66:1293–8.
[15] Tamashiro KLK, Wakayama T, Blanchard RJ, Blanchard DC, Yanagimachi R. Postnatal growth and behavioral development of mice cloned from adult cumulus cells. Biol Reprod 2000;63:328–34.
[16] Walker SK, Hartwich KM, Seamark RF. The production of unusually large offspring following embryo manipulation: concepts and challenges. Theriogenology 1996;45:111–20.
[17] Lai L, Hawley RJ, Park KW, Cheong HT, Greenstein JL, Im GS, et al. Production of alpha (1,3) galactosyltransferase-knockout pigs by nuclear transfer cloning. Science 2002;295:1089–92.
[18] Lai L, Park KW, Cheong HT, Kuhholzer B, Samuel M, Bonk A, et al. A transgenic pig expressing the green fluorescent protein produced by nuclear transfer using G2/M stage fibroblasts as donor cells. Mol Reprod Dev 2002;62:300–6.
[19] Park KW, Lai L, Cheong HT, Cabot RC, Sun QY, Wu G, et al. Mosaic gene expression in nuclear transfer-derived embryos and the production of cloned transgenic pigs from ear-derived fibroblasts. Biol Reprod 2002;66:1001–5.
[20] Lai L, Im G-S, Liu Z, Hao Y, Samuel M, et al. Comparison of in vivo developmental competency of cloned pig embryos derived from three different activation strategies, 2004. Proceeding from the 37th Annual Meeting of the Society for the Study of Reproduction; August 1–4, 2004. Vancouver, British Columbia, pp. 212 abstract 520.
[21] Carroll J, Daniel J, Keisler D, Matteri R. Non-surgical catheterization of the jugular vein in young pigs. Lab Anim 1999;33:129–34.
[22] Lanza RP, Cibelli JB, Faber D, Sweeney RW, Henderson B, Nevala W, et al. Cloned cattle can be healthy and normal. Science 2001;294:1893–4.
[23] Chavatte-Palmer P, Heyman Y, Richard C, Monget P, LeBourhis D, Kann G, et al. Clinical, hormonal and hematologic characteristics of bovine calves derived from nuclei from somatic cells. Biol Reprod 2002;66:1596–603.